Promises and Perils of Lycopene/Tomato Supplementation and Cancer Prevention

Executive Summary Report[1,2]

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Greetings and Opening Remarks

Cindy Davis, Division of Cancer Prevention, National Cancer Institute; John Milner, Division of Cancer Prevention, National Cancer Institute; Paul Coates, Office of Dietary Supplements, National Institutes of Health; Joseph Spence, Agriculture Research Service, U.S. Department of Agriculture

Dr. Cindy Davis welcomed the participants and expressed appreciation for their willingness to share their time and views. She stated that the goals of the workshop were to critically evaluate the epidemiological, preclinical, and clinical evidence related to lycopene/tomato consumption and cancer prevention; to identify typical exposures and metabolomic responses; and to identify possible adverse consequences of lycopene consumption. She indicated the discussions at the end of each session will assist in critically evaluating the current findings and help to identify research gaps, and the workshop's final discussion will focus on setting research priorities. Dr. Davis thanked the sponsors: the Division of Cancer Prevention (DCP),[4] the Center for Cancer Research, and the Division of Cancer Epidemiology and Genetics at the National Cancer Institute (NCI); the Office of Dietary Supplements (ODS) at the National Institutes of Health (NIH); and the Agricultural Research Service (ARS) at the U.S. Department of Agriculture (USDA) and the program committee members for making this workshop possible.

Dr. John Milner welcomed participants on behalf of himself and Dr. Peter Greenwald, the director of the Division of Cancer Prevention. Dr. Milner stated that a major goal of this workshop is to evaluate the quality of the science that is currently available about lycopene/tomatoes and health. He questioned whether the evidence is adequate to justify consumer beliefs. He indicated this workshop is truly a team effort; an NIH "enterprise activity" that involves many different groups with a common focus. He indicated that at a minimum this workshop will yield an Executive Summary that will highlight research gaps and possible directions for use in fostering research in this area. It is possible that a Request for Applications (RFA) or Program Announcement (PA) may arise from presentations and discussion occurring during this workshop. He noted, however, that much of the funded research at NIH is investigator initiated independent of RFAs and PAs. Dr. Milner summarized the "3 Ds" of research: *discovery* (identifying sites of action and their physiological significance), *development* (incorporating basic science into a clinical situation), and *delivery* (relaying information to the public). In nutrition the delivery phase often precedes discovery and development, sending mixed messages about the state and relevance of information. He mentioned that the term "nutritional preemption" might be useful as a strategy to identify target populations who respond to certain foods or food components, including vulnerable populations that might be put at risk by exaggerated intake.

Dr. Paul Coates expressed his pleasure to cosponsor this workshop with the USDA and NCI. Although the ODS does not have direct granting authority, it can collaborate with partners in many areas and has been doing so in a variety of ways. Dr. Coates stated that the gaps in knowledge might be

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[2] A prepublication version of this manuscript is posted on the National Cancer Institute, National Institutes of Health website: http://www3.cancer.gov/prevention/lyco/execusum.html.

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[4] Abbreviations used: APCI, atmospheric pressure chemical ionization; ARS, Agricultural Research Service; BPH, benign prostrate hyperplasia; DCP, Division of Cancer Prevention; EPIC, European Prospective Investigation into Cancer and Nutrition; HGPIN, high-grade prostatic intraepithelial neoplasia; IEB, intermediate endpoint biomarker; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; LC, liquid chromatography; MRI, magnetic resonance imaging; MS, mass spectrometry; NCI, National Cancer Institute; NHANES, National Health and Nutrition Examination Survey; NMU, *N*-methyl-*N*-nitrosourea; NOAEL, no observed adverse effect level; ODS, Office of Dietary Supplements; PA, Program Announcement; PPAR, peroxisome proliferator-activated receptor; PSA, prostate-specific antigen; RARE, retinoic acid response element; RFA, Request for Applications; SEB, surrogate endpoint biomarker.

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wider in supplements than foods; a response to a food does not necessarily correlate with efficacy or health effects in a supplement. Research on the delivery system of supplements for promising bioactive factors should be supported and encouraged. The ODS plans to continue collaborating with the NCI and others to support research and develop new initiatives to help bridge the gap between promising food sources of bioactive factors and bioactive factors delivered in supplement form.

Dr. Jospeh Spence stated that the USDA's ARS was also pleased to cosponsor this workshop, which is aptly named (i.e., the promises and perils of lycopene). The USDA has had a long-standing interest in phytonutrients. For more than a decade, USDA investigators have measured compounds in foods and have tried to develop evidence that the compound is bioactive and beneficial. Plant breeders have requested information on how to modify fruits and vegetables to improve nutritional quality and provide additional health benefits to consumers. However, today the research is not adequate to support a claim that a tomato with increased lycopene will prevent cancer. Despite such concerns, several plant breeders have developed high-lycopene tomatoes; but this may be modifying the amounts of other dietary components. Breeders could be enhancing the amount of one beneficial component and either reducing the amount of another beneficial component or increasing the amount of a detrimental component. The outcome of this workshop, filling the gaps in knowledge regarding the health effects of lycopene, will be critical to deciding the direction of future research.

SESSION 1: HOW PERSUASIVE IS THE EPIDEMIOLOGIC EVIDENCE SUGGESTING A ROLE OF LYCOPENE/TOMATOES IN CANCER PREVENTION?

Session Moderator: *Christine Swanson, ODS, NIH*

What Do Epidemiologic Studies Suggest about Lycopene and/or Tomatoes as Modifiers of Prostate, Lung, or Colon Cancer Risk?

Dr. Edward Giovannucci, Harvard University

Dr. Giovannucci summarized the epidemiological literature on tomatoes, lycopene, and cancer (1). The epidemiologic evidence falls into 4 overlapping categories: retrospective case-control studies, prospective cohort studies, plasma-based studies, and questionnaire-based studies. Etminan et al. (2) conducted a meta-analysis of 11 case-control and 10 cohort studies based on either plasma or dietary lycopene. They found *1*) a significant inverse association between prostate cancer and serum lycopene (but not raw tomato intake, lycopene intake, or cooked tomato intake) in the case-control studies, and 2) a significant inverse association between prostate cancer and raw tomato intake, lycopene intake, cooked tomato intake, and serum lycopene in the cohort studies and cohort/case-control studies combined, with a stronger inverse association in the cohort than combined group. Dr. Giovannucci concluded, from the work of Etminan et al. (2) and others, that a moderate inverse association is observed between tomato products and prostate cancer in most prospective and plasma-based studies of lycopene. This association is unlikely to be caused solely by chance or bias and persists in most multivariate analyses, but residual confounders cannot be eliminated.

Numerous case-control studies have reported inverse associations between tomato intake and risk of lung and gastric cancers, as well as several other cancers. Dr. Giovannucci stated that the studies of tomatoes, lycopene, and other cancers are suggestive, but not always consistent. In addition, few of the studies separated tomatoes from other fruits and vegetables, and almost no prospective or plasma-based studies have been conducted.

Additional conclusions are that *1*) most dietary-based case-control studies ($n = 7$) do not support an association with lycopene; *2*) most dietary-based cohort studies ($n = 4$), plasma-based cohort studies ($n = 6$), and plasma-based case-control studies ($n = 2$) support a 25 to 30% risk reduction; *3*) this risk reduction is observed at lycopene intakes of ~10,000 μg/d or blood concentrations of ~0.75 μmol/L; *4*) these associations are relatively modest, but if causal they are important because a single measure of diet and plasma would underestimate the true association; 5) the epidemiologic results apply only to tomato products (supplemental lycopene has not been studied); 6) although there is no obvious source of confounders, this cannot be excluded entirely; and 7) future research should include epidemiologic studies in diverse settings (e.g., non-United States), randomized intervention studies (prediagnostic and postdiagnostic), and studies to identify genetically susceptible groups.

Discussion. It was suggested that, because the association is strongest in the older group in which there is more obesity, the insulin-like growth factor-1 (IGF) might be involved. Dr. Giovannucci replied that, although the results are preliminary, he observed that IGF was associated with higher risk of prostate cancer in general, particularly for the more advanced cancers. When divided by grade, IGF was associated with better differentiated (lower grade and less advanced) cancers. He further speculated that cancers that are poorly differentiated, high grade, and more advanced at diagnosis may be insensitive to endogenous or exogenous factors. In contrast, those that are better differentiated may be more susceptible to factors such as IGF. Lycopene and tomatoes also appear to be associated with better differentiated, less advanced cancers.

One participant asked whether there is a potential interaction between lycopene and any other antioxidant that may contribute to the risk-reduction profile. Dr. Giovannucci replied that, if lycopene acts as an antioxidant, interactions would be expected with selenium and vitamin E. When a high-lycopene, high-tocopherol, high-selenium group of a population was compared to a group that was low in all of those factors, a highly significant, 10-fold difference in relative risk was observed in men with a specific variant of the manganese-dependent superoxide dismutase gene. This study should be replicated, but it suggests the presence of more susceptible groups.

African-American men have a heightened risk of prostate cancer. One participant stated that the results of her case-control study showed that lycopene was inversely associated with risk in Caucasian and African-American men. It has been found that African-American men eat fewer tomatoes, and their blood lycopene levels are lower. Dr. Giovannucci responded that only about 1% of his study population was African American; however, even in such a small sample, he observed a significantly enhanced risk for prostate cancer among African-American men. He indicated that the African-American men in the study had a slightly lower intake of tomato products, but this study did not have the power to tease out the risk to this population.

Another participant asked whether there is any evidence that fat intake is a determinant of the response to lycopene or tomato products. Dr. Giovannucci replied that this issue has not yet been studied from an epidemiological perspective, but it could be in the future.

What International Perspectives Does the EPIC Study Provide about Lycopene/Tomatoes versus a "Mediterranean Diet" for Cancer Prevention?

Dr. Elio Riboli, International Agency for Research on Cancer

Dr. Elio Riboli described the European Prospective Investigation into Cancer and Nutrition (EPIC) study (3). It is a large-scale, multilingual, multicultural study that takes advantage of the significant variation from northern to southern Europe in diet, lifestyle, and cancer risk. Anthropometric measurements were taken and lifestyle data (e.g., diet, physical activity, tobacco and alcohol use) were collected using a questionnaire. All of the 521,000 EPIC subjects answered a list of questions about usual diet (150–300 foods) designed to relate to cancer risk. In addition, 7% of EPIC subjects (37,000 subjects) answered a more detailed, computerized list of questions (3000 foods and >700 recipes/country) designed to calibrate dietary measurements among countries. This 24-h diet recall method is now being used in several European countries for nutrition and health surveys.

Baseline data (subject recruitment, questionnaire data, anthropometry data, blood/DNA collection, and biorepository) were collected from 1993 to 2003, follow-up data (cancer diagnosis, vital status, causes of death, and changes in lifestyle) were collected from 2000 to 2004, and the etiological studies to link the follow-up and baseline studies began recently. In addition, a cross-sectional study was conducted within EPIC using 3100 subjects (100 men and 100 women in each relatively homogeneous geographic region, stratified by age) to measure different lifestyle and metabolic factors (e.g., carotenoids, vitamin C, fatty acids, lycopene).

In the EPIC database, the correlation between estimated tomato consumption (raw, cooked, or industrially manufactured) and lycopene serum level was relatively strong at the ecological level ($r = 0.50–0.70$) but was weak at the individual level ($r = 0.10–0.20$). If lycopene is the variable of interest to be related to cancer risk, then serum lycopene level rather than estimated tomato consumption should be used. If serum lycopene level is associated with reduced prostate cancer risk, then dietary advice on tomato consumption should allow for individual variations in bioavailability, absorption, and metabolism.

Discussion. One participant asked whether the lycopene isomers are added together or considered separately in the EPIC study. Dr. Riboli responded that his method produces a 3-dimensional, mountain-shaped image of the isomers of lycopene and other carotenoids. The lycopene peaks, which are quite distinct from surrounding peaks in the 3-dimensional representation, are added together. He again noted that the blood measurement data are reasonably good but the dietary measurement data are not.

In the Physicians' Health Study, one-half of the participants received a β-carotene supplement, and the other half received a placebo. In the placebo group, a strong inverse relationship was observed between serum lycopene and cancer risk. No such trend was evident in the β-carotene group. In a group of participants with low lycopene, however, β-carotene appeared to have a protective effect. One interpretation of these data is that there is a plateau or ceiling, and populations that already are receiving a high level of antioxidants or other nutrients through diet or supplements may be at or near the plateau of protection. Dr. Riboli stated that he is measuring 7 different carotenoids to calculate a total carotenoid measure. Perhaps the "possibility of absorption" and the "possibility of storage" of the different carotenoids are limited. In effect, there may be competition between β-carotene and lycopene, which should be investigated at the experimental, rather than at the epidemiological, level.

A participant asked whether the correlation between prostate cancer mortality rates and lycopene levels has been studied in the EPIC study. Dr. Riboli replied that a negative correlation between prostate cancer incidence and tomato consumption/lycopene levels was found at the ecological level, but not at the individual level.

What Are the Future Needs within the Epidemiological Domain That Relate to Lycopene?

Dr. Alan Kristal, Fred Hutchinson Cancer Research Center

Dr. Kristal provided a review of the published observational research; described the limitations and future research directions of lycopene exposure measurements, prostate cancer epidemiology, and genetic variability; and critiqued past and ongoing human trials studies with intermediate biomarker endpoints (4).

He concluded that the results from the strongest epidemiologic studies (i.e., prospective studies with cooked tomatoes, dietary lycopene, or serum lycopene) appear mixed. Many of the studies are too small to detect modest effects, and the associations may be restricted to population subgroups. The most relevant lycopene exposure measurement is probably prostate tissue lycopene concentration, but only serum and diet concentrations can be measured in epidemiologic studies. Consumption of lycopene and high-lycopene foods appears to correlate poorly with serum lycopene. Single-dose lycopene feeding studies do not reflect long-term intake, and inferences from single-dose studies may not be accurate as to the lycopene that is or is not bioavailable. Thus, the best measure of prostate tissue lycopene exposure is likely to be multiple serum lycopene measures collected over time.

The more that is learned about prostate cancer, the more difficult it may be to study the disease. Prostate-specific antigen (PSA) is not a sensitive screening test for prostate cancer; some men with prostate cancer have "normal" PSA levels. In addition, PSA screening guidelines may mask the association between lycopene and cancer risk. Contemporary epidemiologic studies of prostate cancer require detailed information on screening history, stage, and grade. The Surveillance, Epidemiology, and End Results (SEER) cancer registries may obscure grade classifications by grouping Gleason scores as 2–4, 5–7, and 8–10. A Gleason score of 7 is common and clinically high grade.

Key questions that need to be answered include the following: Do the effects of lycopene vary with genetic characteristics? For example, lycopene may have a substantial chemopreventative effect only among men with a high susceptibility to oxidative DNA damage. Research in this area is currently speculative and it would require large sample sizes to investigate the question fully. Another question to consider: Has the misinterpretation of widely quoted pilot studies for clinical trials misled both scientists and the public? Clinical trial studies should meet the same critical standards as epidemiologic studies. For example, an experimental study without a control arm would not be informative about the intervention effect. Small clinical trials with intermediate biomarker endpoints require validation of endpoints and rigorous design and execution. In addition, a decision to begin a prevention clinical trial requires much more scientific data.

In summary, Dr. Kristal concluded that *1*) epidemiologic studies are mixed but not generally supportive, and human clinical trials to date are not informative; *2*) definitive epidemiological studies will require a better assessment of lycopene

exposure, a better characterization of prostate cancer, and larger sample sizes; and 3) definitive clinical trials will require validation of intermediate endpoints and rigorous design and execution. There is considerable room for improvement in epidemiologic studies, particularly with respect to exposure assessments, control for effect modification (e.g., gene interactions), and confounding (e.g., PSA).

Discussion. A participant noted that data in animals and humans suggest that there is a maximal lycopene uptake into mucosal cells from a single meal. Lycopene and other carotenoids generally remain long enough for an additional amount to come in from a second meal. There also is a limited amount of lycopene that can move from the mucosal cells into lymph at any one time, which is also dependent on the amount of fat consumed. Thus, although there appears to be a maximal dose level in tissues, there may be continued residual uptake from additional meals because of delays in absorption, recirculation, and metabolism. One participant hypothesized that, with multiple doses, there could be more accumulation of lycopene in tissues that may not be reflected in the plasma. Dr. Kristal agreed that, if the serum level reaches a plateau, there still might be accumulation in tissues. Little research has been done on this topic.

Another participant commented that although there is a need for placebo controls, particularly in biomarker studies, it would be more difficult to implement them with food-based interventions than with supplements. Dr. Kristal explained that he is less concerned with the issue of placebo control than with having a "meaningful comparison group" from which to make some inference. A placebo is optimal, but it is difficult to use placebos with food studies.

A participant asked what is meant by a "validated" biomarker for clinical trials in prostate cancer. Currently, it is not clear whether even biopsy-proven prostate cancer is a predictor of morbidity or mortality. Gleason grade or stage could be as close to a validated biomarker as is possible at this time. Dr. Kristal stated that the biomarker should have "face validity" and make sense. Meaningful endpoints should be identified. The use of intermediate biomarkers is an extremely complicated issue.

A participant asked whether surrogate cells might provide some clues (e.g., buccal cavity cells or exfoliated cells). Dr. Kristal replied that an exfoliated cell could be a better measure of lycopene in prostate or other tissue than serum. This is an interesting point that should be investigated further.

Group Discussion 1: Defining Research Gaps and Setting Research Priorities

Moderator: *Regina Ziegler, Division of Cancer Epidemiology and Genetics, NCI*

A suggestion was made to examine bound rather than total PSA in epidemiological studies to provide a more specific indicator of prostate health. Another participant replied that free PSA alone is no more useful than total PSA; the utility in predicting prostate cancer is the combination of total and free PSA. PSA is thought to be elevated in prostate cancer because of leakage through the faulty microcirculation of the tumor. Unless lycopene can repair that microcirculation, there is no reason to believe that lycopene treatment will lead to a reduction in PSA. Another participant stated that there are 2 explanations in addition to leaking for a reduction in PSA: *1*) a decrease in tumor cell number (less cells to produce PSA), or *2*) a nutritional substance such as soy or lycopene blocking tumor growth or PSA production (the cells produce less PSA). A counterpoint was offered that prostate cancer cells produce less PSA than normal cells, not more, because they are highly undifferentiated, and PSA is a differentiation marker for prostate cancer. Another participant suggested that both viewpoints are correct: prostate tumor cells produce less PSA per volume than epithelial cells; however, the driver of the PSA level is the tumor. Lycopene or another nutritional component may have some effect on the biology of the tumor, decreasing PSA. A comment was made that an overemphasis on intermediate markers of risk such as PSA has led to misinterpretation of cancer prevention research findings. A large proportion of prostate cancer is diagnosed in subjects with normal PSA levels; thus, clinical trials should be conducted on prostate cancer rather than an intermediate marker such as PSA.

Although data are limited, some evidence suggests that lycopene absorption is depressed in the elderly. One study found that age is inversely associated with plasma lycopene levels, even after adjusting for dietary intake. Suggestions were made to age-stratify epidemiological studies and to focus on some observational epidemiological studies within the context of screening trials or trials in which the diagnostic bias can be removed. In the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), for example, subjects are screened according to the same schedule. Alternatively, the trial could be conducted in a country without the same screening schedule.

The epidemiologic studies of circulating lycopene levels seemed to show more evidence of a protective effect than the studies of tomatoes or dietary lycopene. Circulating lycopene level is determined by tomato intake as well as by absorption and bioavailability. One participant asked, given our knowledge of the role of different subtypes of fat in prostate cancer and the effect of cholesterol subtypes on lycopene bioavailability, whether the observed protective effect could be more related to some aspect of fat metabolism than to simple lycopene intake. That is, are there factors that determine circulating lycopene level other than tomato intake that might be more important than previously thought in terms of the protective association? The comment was made that olive oil is consumed with tomatoes more often in the Mediterranean region. Olive oil may have some protective effect apart from lycopene, adding another confounding factor. The absorption of lycopene with different types of fat has not been investigated and deserves further study.

A question was asked whether or not NCI might facilitate collaboration across epidemiological studies to expand the specific population under examination. A suggestion was made to obtain funding for the collection of biological materials in ongoing studies (e.g., cohort studies without blood). There has been recent interest in collecting samples to allow for data-mining activities. Another recommendation was to continue standardization of the biorepositories. However, another participant noted that it may be difficult to obtain funding for studies to stabilize biological specimens.

One participant suggested as a research gap the lack of a consistently used, comprehensive, food frequency questionnaire that collects data on all food products containing antioxidants as well as on the potential polyphenols, lipids, and supplements that might interact with those antioxidants. A single, validated instrument is needed that captures the complete picture. In addition, serum levels only provide a snapshot of what patients absorb and metabolize. Historical data from biological samples are not being captured in epidemiological studies across and among countries. Another participant dis-

agreed, advocating the use of several good instruments to prevent the bias of a single instrument.

SESSION 2: WHAT IS TYPICAL LYCOPENE EXPOSURE AND METABOLOMIC RESPONSE?

Moderator: *Dr. Beverly Clevidence, ARS, USDA*

How Can the Metabolomic Response to Lycopene (Exposures, Durations, Intracellular Concentrations) in Humans Be Adequately Evaluated?

Dr. Steven Schwartz, Ohio State University

Physical and thermal treatment of foods causes degradation of plant cell structural constituents. Thermal processing disrupts the carotenoid protein complexes, and the inactivation of oxidizing enzymes results in less degradation and greater stability of carotenoids. These changes result in enhanced uptake and efficiency of lycopene absorption. For example, lycopene is more bioavailable from processed tomato paste than from fresh tomatoes. Dr. Schwartz's preliminary data suggest that *1*) the physical state of lycopene in the plant tissue (crystalline, amorphous, or soluble within the lipid phase) affects uptake from the matrix, which influences blood and tissue levels; *2*) the concentrations of lycopene in blood and tissues can be altered by the consumption of commercial tomato products within relatively short intervention periods; *3*) food processing, through physical and thermal treatments, has the potential to enhance absorption by disrupting the plant tissue matrix, disassociating carotenoid-protein complexes, enhancing surface area, and increasing solubility; *4*) structural considerations influencing solubility, molecular size, and geometrical isomerization to *cis* lycopene isomers can enhance bioavailability; and *5*) lycopene is absorbed poorly relative to other carotenoids in the diet; however, coconsumed lipid is critical and can enhance absorption from the tomato matrix (5).

Discussion. One participant asked whether lutein levels have been measured in the tangerine variety of tomato and whether the isomers are a result of lycopene oxidation. Dr. Schwartz replied that the levels of xanthophylls such as lutein and zeaxanthin have not been measured in the tangerine variety, but the lutein level in most tomatoes is low. The same low lutein level is expected in the tangerine variety; however, significant levels of 9-*cis*-β-carotene have been observed. Because the isomerase is missing, there must be some retention of the isomeric forms (from lycopene through the cyclization reactions to β-carotene) further down the biosynthetic pathway. Dr. Schwartz added that it is not known whether the isomers are a result of oxidation. Lycopene and other carotenoids might isomerize with oxidative stress, but the evidence is not definitive at this point.

Another participant asked about the sources of tomato that were used in the studies conducted by Dr. Schwartz. Dr. Schwartz replied that the sources included a tomato salsa (with no fat but avocado) and a commercial tomato sauce (formulated with some lipid). He indicated that the coconsumed lipid in the tomato sauce most likely accounts for the rise in plasma levels compared to the groups that consumed V8 juice or tomato soup.

Dr. Schwartz noted that homogenization appears to affect β-carotene absorption more than lycopene absorption, but the reverse was true for fat (i.e., fat had more of an effect on lycopene absorption than on β-carotene absorption). One participant asked about the consistency of these findings across studies. Dr. Schwartz responded that β-carotene already is associated with lipid inside the plastoglobulin membrane of the tomato tissue, whereas lycopene is not. Thus, fat influences lycopene absorption more than β-carotene absorption by providing the lipid necessary for enhanced lycopene solubility and uptake. β-Carotene in the tomato matrix already is somewhat soluble; therefore, fat may not have as significant an effect. These results should be consistent with other studies.

What Are Typical Lycopene Intakes?

Dr. Marisa Porrini, University of Milan

Evidence suggests that high consumption of tomato products or lycopene is associated with a significantly lower risk of cancer. It is difficult to identify "typical" lycopene intakes and to determine the dietary levels necessary to achieve a biological response (6). Data on lycopene intake differ considerably among countries, and among populations within the same country. Factors contributing to these differences include the method used to estimate food intake, the food database considered, and the natural variability of the lycopene concentration in food. The bioavailability of lycopene varies greatly with food source and appears to depend on *1*) the release of lycopene by technological processing, including the disruption of tissue structure and cell walls; *2*) thermal weakening and dissociation of lycopene-protein complexes; *3*) dissolution and/or dispersion of crystalline lycopene aggregates; and *4*) heat-improved extraction of lycopene into the oil phase.

Because of the problems involved in quantifying tomato/lycopene intake, the measurement of lycopene concentration in blood may provide a useful link with dietary exposure in epidemiological studies. However, several factors may influence blood lycopene levels, including plasma cholesterol and triglycerides, marital status, age, alcohol intake, body mass index, energy intake, supplement use, vegetable and fruit intake, and vitamin E intake. However, a large proportion of the variance in plasma lycopene concentrations remains unexplained. Additional factors affecting the lack of correlation between intakes and blood concentrations include the timing of blood collection in relation to dietary assessment, recent lycopene intake, changes in absorption related to age and genetics, and the individual absorption capacity. To conclude, a better understanding of dietary intakes within and among countries is needed. The results available from intervention studies seem to suggest that the regular intake of small amounts of tomato products providing about 6–8 mg lycopene is sufficient to increase cell resistance to DNA oxidative damage. However, it should be mentioned that apart from lycopene, other potentially protective compounds are present in tomato products. Consequently, dietary reccomendations should consider tomato intake more than lycopene intake.

Discussion. In many of the published epidemiological studies, the sample size is small and thus does not necessarily represent the entire population. The mean intake value can be misleading (i.e., the mean and median values can differ greatly). It may be useful for epidemiologists to also consider the range of intakes or specific biomarkers. Other problems with the literature are that the methods used to assess intake vary and are not always adequately described and that many studies were not designed to focus on lycopene per se.

One participant stated that data for the United States from the National Health and Nutrition Examination Survey (NHANES) were published in Appendix C of the National Academy of Sciences Report (http://www.nap.edu/catalog/9810.html) (7). The data are categorized by age, sex, and percentile, and extreme skewing is observed in the distribution. A

recommendation was made to use the NHANES data as a reference for the United States.

One participant asked how long the oxidative protection of DNA persists. Dr. Porrini replied that there is no definitive answer. The participant stated that the length of oxidative protection could be an important question for future research.

There still is debate within the scientific community as to the benefit of tomato products as a whole. The data seem to be weaker with respect to pure lycopene. One participant suggested that, when making recommendations, the target should be tomato product consumption within the context of a healthy diet, rather than pure lycopene, since it may not be accurate to ascribe all of the protective effects to lycopene.

How Do Nutritional and Hormonal Status Modify the Bioavailability, Uptake, and Distribution of Different Isomers of Lycopene?

Dr. John Erdman, University of Illinois-Urbana-Champaign

Metabolic or disease factors unrelated to food intake that may affect lycopene absorption and metabolism include the following: *1*) fat malabsorption syndromes and intestinal parasites; *2*) some hypolipidemic drugs; *3*) liver or kidney disease; *4*) hypothyroidism or hyperthyroidism; *5*) anorexia nervosa, bulimia, or weight loss; *6*) stage of estrous cycle; and *7*) androgen status (8).

The isomer composition of lycopene (*cis* versus *trans*) in tomato products changes with processing. These differences in isomer composition may have a metabolic effect, affecting factors such as the ability to influence health outcomes, antioxidant properties, susceptibility to oxidation, specificity of enzymatic cleavage and metabolism, and differential uptake and accumulation in tissues. The literature suggests that the differential accumulation of lycopene in tissues may be *1*) correlated to the number of low-density lipoprotein receptors, *2*) dependent on the amount of fat in tissues, *3*) preferentially taken up by reproductive tissues, *4*) dependent on the "metabolic rate" of tissues, *5*) dependent on binding or transport proteins within tissues, *6*) caused by differences in *cis* versus *trans* uptake and metabolism, or *7*) related to "needs" of the tissues.

Regarding hormonal status, androgen depletion or 20% food restriction increases hepatic lycopene and vitamin E accumulation. Thus, higher androgen status or greater energy consumption may stimulate lycopene metabolism and degradation. Data on the effects of the stage of the estrous cycle on lycopene metabolism are varied, and many of the metabolic effects are not yet well understood.

Future research opportunities include an investigation of the following: *1*) why *cis* isomers are differentially taken up by tissues, *2*) why tomato carotenoids (lycopene, phytoene, and phytofluene) are either taken up or accumulate in a differential manner tissue to tissue, and *3*) why androgen levels appear to affect lycopene accumulation. In addition, work should continue on identifying important tomato components other than lycopene (e.g., phytoene and phytofluene).

Discussion. There is evidence that lycopene stability and *cis* and *trans* isomer levels may be related to the thermodynamic stabilities of the various lycopene forms. Lycopene in solution rapidly isomerizes to form a mixture of isomers (the half-life of lycopene in solution is on the order of hours). It appears that lycopene is stabilized in the chloroplast of the tomato but also in human cells (where lycopene persists for days, not hours). One participant suggested that lycopene might be localized in the lipid bilayers of cells. Dr. Erdman replied that little is known about lycopene localization within cells. The data have shown that the size of the bilayers, as well as the polarity of lycopene, would make it impossible for lycopene to exist perpendicular to the membrane. Lycopene may exist between the bilayers or be associated with lipid droplets or lipids within cells.

A participant asked about the evidence that *cis* isomers are preferentially absorbed, given the short time period (minutes) required for isomerization. Once the lycopene leaves the plant matrix in the gut (where it is stabilized), the lycopene must either come out of solution and quickly isomerize or remain in lipid as it is absorbed. Dr. Erdman replied that artificial micelles, similar in size to micelles of the human gut, were used to explore the solubility of all-*trans* versus *cis* isomers. *Cis* isomers were found to preferentially accumulate in those micelles. The difference between the small intestine contents and mucosal cells in terms of percentage of *cis* also suggests that *cis* forms are absorbed preferentially.

A participant commented that, with regard to hormone status, his laboratory observed higher accumulations of lutein and zeaxanthin in female versus male Japanese quail. His laboratory also is working on the NHANES analysis, and their data will include phytoene and phytofluene for the general human population.

An immediate precursor to lycopene in biosynthesis is phytofluene, and the immediate precursor to phytofluene is phytoene. The structures of phytoene, phytofluene, and lycopene differ by only 1 or 2 saturated double bonds. One participant asked why these small structural differences would have such a significant effect on uptake of the compounds. Dr. Erdman speculated about the existence of preferential transport proteins.

Another participant speculated that the higher accumulation of lycopene in the liver could be for storage lycopene, as is the case with other antioxidants. Dr. Erdman stated that he dislikes the word "storage" because a mechanism that blocks movement is implied. He noted that the liver also transports newly absorbed carotenoids out of the liver, so it is possible that there is no storage mechanism keeping lycopene in the liver.

Another participant commented that castration is usually associated with changes in lipid metabolism. He asked whether the lycopene accumulation could be explained by the fact that castration modifies lipid metabolism, perhaps via the proportionally higher estradiol concentration. Dr. Erdman replied that this is plausible, but when the testosterone implants are added, lycopene level returns to normal.

How Can Pharmacokinetic Modeling Be Used to Understand Lycopene Disposition and the Potential Role of Lycopene in Cancer Prevention?

Dr. Janet Novotny, USDA, Beltsville, MD

One of the goals of nutrition-oriented scientists is to provide information that can be used to develop recommendations for intakes of nutrients and health-providing compounds. To move forward with this goal, answers are needed to the following questions: *1*) What percentage of a compound is absorbed? *2*) How much of a compound reaches a target tissue, and how long does the compound remain in that target tissue? *3*) What are the pool sizes? *4*) How fast is a compound irreversibly utilized? The tools of mathematics (mathematical modeling, compartmental modeling, pharmacokinetic modeling, and physiologically based pharmacokinetic modeling) can be used to unravel complex biological systems (9). A lycopene kinetic model has been developed in which the different compartments are connected by first-order linear differential

equations. If the model's prediction of how a lycopene dose will affect blood matches subject data and is physiologically sensible, then the model is considered a good predictor of how the system works. If the model's blood prediction is not a good match, then the rate constants or modeling structure are altered. The lycopene kinetic model was determined to be a good predictor of plasma response and was used to predict a decline in absorption efficiency with increasing dose. Another useful aspect of mathematical modeling is that it allows the monitoring of tissue lycopene levels.

Model simulations were expanded to examine different dosing regimes. Distributed dosing of lycopene was found to be more effective than single daily dosing when the distributed doses were assumed to be independent (which may not be true). The model showed good agreement with daily dosing studies. The adjustment of treatment bioavailabilities brought the model and study values closer together; thus, pharmacokinetic modeling may be useful in predicting treatment bioavailabilities. Research also was done on how dose response extends to chronic intakes. That is, does the diminishing absorption efficiency inhibit the ability of chronic doses to affect plasma lycopene levels? Diminishing returns were observed with higher lycopene doses. The data show a larger boost in plasma lycopene when the dose is increased from 40 to 60 mg/d than from 20 to 40 mg/d, suggesting 2 different absorption mechanisms.

Interindividual variability could be incorporated into model populations by employing the Monte Carlo technique. In addition, modeling could be used to identify differences in metabolism among populations. Although this technique has not been used with lycopene or any other carotenoid, specific calcium metabolism pathways that differ during rapid versus slow bone accretion have been identified by mathematical modeling. Mathematical modeling across species—a well-validated technique common in the toxicology field—is achieved by including species-specific parameters and could be useful in understanding lycopene handling by the prostate. As modes of lycopene action are elucidated, modeling of lycopene-sensitive processes (rather than simply lycopene disposition) may prove valuable.

With respect to lycopene and cancer, future physiologically based pharmacokinetic modeling should investigate the following: *1*) the effect of dose on plasma and tissue response, *2*) different dosing patterns, *3*) doses between 0 and 30 mg, *4*) tissue accumulation and elimination, *5*) modeling of lycopene-influenced processes (when the appropriate information becomes available), and 6) lycopene disposition by different populations. In summary, there are many opportunities for modeling to unravel key issues surrounding lycopene disposition and its potential role in the prevention of prostate cancer.

Discussion. One participant asked whether the modeling or the study data predicted that there is a different absorption mechanism beyond 30 mg. Dr. Novotny responded that this finding was based on clinical study data (information of intake and serum levels but not on excretion). Another participant commented that, with regard to the agreement between the simulated level and the actual level in repeated dosing studies, the width and variability of the range is surprising (i.e., the model would appear to agree with the actual data for a long time, based on Dr. Novotny's criteria). Dr. Novotny suggested using Monte Carlo simulations to model populations rather than individuals. A comment was made that a dose of 30, 60, or 120 mg most likely will persist in the intestine for a long time, with the ability to be absorbed days later (possibly from mucosal cells or the lymphatic system). Modeling will be difficult because there may be unpredictable interchanges among the different pools.

A participant asked about the reliability of the data, considering interindividual differences in body stores. There were no studies done with isotopically labeled lycopene to try to differentiate the newly administered dose from what already was in the body. Dr. Novotny replied that it would be helpful to know whether the lycopene in the plasma was supplied from the diet or by the liver or small turnover pool. However, the fractional standard deviations all were <60% and often <20%, providing more confidence that the lycopene was supplied by the dose and not the tissues.

Group Discussion 2: Defining Research Gaps and Setting Research Priorities

Moderator: *Johanna T. Dwyer, ODS, NIH*

Tomatoes contain almost 300 compounds, and it might prove beneficial to study tomato compounds other than lycopene, such as tomatine, as well. Although the tomatines are considered to be toxic, evidence suggests that they may also be cancer protective. Tomatines tightly bind cholesterol, and, because there is cholesterol in cell membranes, tomatines are very disruptive in cell culture. Dr. Bowen replied that her cell culture experiments with tomatine versus lycopene yielded viable results, and that differences in viability were not observed. It was also suggested that future research be directed toward investigation of 2 additional compounds abundant in tomatoes—pectin and oligosaccharides. Another recommendation was that the synergistic activity among lycopene, phytoene, phytofluene, and β-carotene be studied. When 2 of these compounds are present together, at concentrations where alone they have no effect, there is a significant decrease in prostate cancer cell proliferation and an increase in apoptosis.

From an epidemiological perspective, total, *cis*, and *trans* isomers of lycopene are highly intercorrelated, in that they are associated with the same outcomes. From a physiological perspective, however, one isomer may be more important than another. Thus, relevant questions were why isomerization is not 100% and what the fate of the *trans* isomers is specifically. Bowen described an experiment in which 5 scenarios were modeled with 10 subjects at different doses: no interconversion between *cis* or *trans* isomers and various fixed conversion percentages or fixed absorption of the percentages were noted. The model that assumed no conversion fit the data fairly well at all doses, although the model with conversion fit the data slightly better. In all of the models, however, *cis* isomers were better absorbed, and *trans* isomers left the system more quickly.

A participant asked whether there is enough lipid in multivitamins to ensure that lycopene is bioavailable. Published data does suggest that the compound in multivitamins is highly bioavailable.

SESSION 3: WHAT HAVE WE LEARNED FROM STUDIES IN MODEL SYSTEMS?

Moderator: *Dr. Susan Percival, University of Florida—Gainesville*

What Has Microarray Analysis Revealed about the Mechanisms of Action of Lycopene in Prostate Tumors?

Dr. Karin Wertz, DSM Nutritional Products

High tomato intake and plasma lycopene levels are associated with a reduced prostate cancer risk. The objective of this

research project was to investigate 2 main questions: *1*) Is lycopene, the main carotenoid in tomato, responsible for the observed effect of tomato consumption? *2*) If so, by what mechanisms does lycopene contribute to the reduced risk of prostate cancer (10)?

The effects of lycopene on tumorous and normal rat prostate tissue were compared. The data suggest that in tumorous rat prostate tissue, lycopene *1*) significantly increased the necrosis rate compared to a placebo group (37 versus 23%, respectively), *2*) reduced androgen signaling, *3*) decreased IGF-I expression, and *4*) downregulated interleukin-6 expression. In normal rat prostate tissue, lycopene *1*) had no effect on prostate growth, *2*) reduced androgen signaling, *3*) decreased IGF-I expression, and *4*) downregulated inflammatory signals.

In summary, lycopene reduced androgen signaling in both normal and tumorous rat prostate tissue. Although the same metabolic pathway was affected, different enzymes were regulated at the transcriptional level, accompanied by downregulation of the same steroid target genes. Both in normal and tumor tissue, lycopene decreased IGF-I expression and downregulated inflammatory signals (with a stronger anti-inflammatory effect in normal prostate tissue than in tumors). Dr. Wertz also discussed some preliminary unpublished data that suggest that lycopene has no systemic influence on androgen signaling and that the local antiandrogen effect is specific to the prostate.

Discussion. A participant asked whether there were other genes that were also affected by lycopene. Dr. Wertz commented that she had presented the most consistent results. Within one pathway, there are often many opposing directions of gene regulation that can be difficult to understand. Dr. Wertz's approach was to group the genes by pathways and metabolic functions, try to identify target genes on those pathways, and determine whether the regulation "fits together."

A participant asked whether there were measures of tumor growth besides necrosis and why such a rapidly growing cell line was used. He also asked about the effects of castration or androgen deprivation on the cell line. Dr. Wertz responded that she did not conduct any tumor histology and that the cell line was recommended by a collaborator who had worked with the model previously. She stated that the MatLyLu officially is an androgen-independent cell line, based on analysis from 20 y ago. It is difficult to explain the downregulation of androgen target genes, however, if the tumor truly is androgen independent. The participant stated that the tumor is classically known to be androgen independent, but this finding could be verified by assessing the growth of the tumor in a castrated animal. The tumor is poorly differentiated histologically, which is consistent with an androgen-independent cell line. He questioned whether a subtle change of 20, 30, or 40% in the expression of an androgen-metabolizing enzyme could bring about a demonstrable change in the growth of such a tumor. Dr. Wertz clarified that the tumor size was not changed. The participant replied that the more rapidly a transplantable tumor grows, the more necrosis is present. When rapidly growing tumors are transplanted, they quickly outgrow their vascular supply, and the central cells undergo necrosis. In the absence of histology, questions about the mechanism remain. Dr. Wertz stated that this research resulted in descriptive data—a readout of what is happening. The data should be fit together to build a working hypothesis. These experiments are not the end, but the start of testing that hypothesis. A comment was made that even androgen-independent cell lines are known to respond to androgen receptor-signaling.

The participant asked whether the entire tumor was homogenized for RNA. If the more necrotic tumors from the lycopene group were treated the same as the less necrotic tumors, there would be a shift in the cells being arrayed. Dying cells might have different gene expression because they were undergoing necrosis. Dr. Wertz responded that she cut out a slice. Such drastic differences in gene regulation between treatment groups are not related to a few percentage points more or less of necrotic cells. In addition, the same signaling pathways were affected in healthy tissue.

Another participant stated that a change in tumor size might be observed with serial magnetic resonance imaging (MRI) and asked whether repeat MRIs were done. Dr. Wertz replied that they did only one MRI.

Insulin-like growth factor binding protein (IGFBP)-3 is known to be a major regulator of IGF-I function. A participant asked whether Dr. Wertz checked for IGFBP-3 expression. Dr. Wertz replied that in this study, IGFBP-3 expression was not found after lycopene supplementation. It may be more important to identify pathways that consistently are regulated in the same direction, rather than trying to identify single genes. The data support the view that something happens to the IGF-I axis.

Dr. Wertz commented that there were 8800 genes on the chips. Nutritional compounds often have effects between 20 and 50% upregulation or downregulation. Thresholds of different stringency should be used, depending in part on the number of chips per group (the more chips, the lower the threshold). The goal is consistency, rather than reading at a sharp threshold. The chip community is moving toward statistical modeling of all the data.

Another participant stated that current research has shown that there is significant diurnal variation in nuclear transcription factors. He asked whether it is valid to investigate a single time point, given the temporal response in nuclear transcription factors, or if multiple time points should be used. Dr. Wertz replied that multiple time points would be better, but it costs more to investigate temporal response.

Can Smoke-Exposed Ferrets Be Utilized to Unravel the Mechanisms of Action of Lycopene?

Dr. Xiang-Dong Wang, USDA, Tufts University

Dr. Wang's talk focused on the effect of the dose of supplemental lycopene and the interaction of lycopene metabolism with cigarette smoke (11). His interest in this area arose because of the conflicting results of β-carotene clinical intervention trials in cigarette smokers (which used high doses of β-carotene and reported increased lung cancer risk) versus the observational epidemiological studies that found that diets high in fruits and vegetables containing carotenoids (but at much lower concentrations than in the intervention studies) were associated with a decreased risk for lung cancer. There are also conflicting reports on the effects of lycopene on lung carcinogenesis in animal studies. An important question that remains unanswered is whether a low dose of lycopene (or its metabolites) provides protection against lung carcinogenesis without increasing the risk of undesirable metabolic by-products (especially in smokers and alcohol drinkers). If so, what are the possible mechanisms? These questions need to be addressed with an appropriate animal model. Ferrets provide an excellent model for studying the chemopreventive effects of lycopene and lycopene metabolites, particularly in the earlier stages of lung carcinogenesis, because they fulfill the following 5 criteria: *1*) absorption and accumulation of intact carotenoids (e.g., β-carotene and lycopene) in a dose-dependant

manner, *2*) conversion of carotenoids into their oxidative metabolites (e.g., β-carotene into vitamin A and lycopene into apo-lycopenoids), *3*) high homology to human genes (e.g., carotene cleavage enzymes, IGF-I/IGFBP-3, p53 tumor suppressor), *4*) lung preneoplastic lesions (e.g., squamous dysplasia and atypical adenomatous hyperplasia) and lung tumor production (e.g., squamous carcinoma and adenocarcinoma), and 5) fearlessness as a study subject, tolerating smoke exposure and blood sampling and providing an ample volume of tissues for analysis.

Epidemiological evidence indicates that increased levels of IGF-I, reduced levels of IGFBP-3, or an increased ratio of circulating IGF-I to IGFBP-3 are associated with an increased risk of developing several common cancers, including breast, prostate, colorectal, and lung. Epidemiological studies provide supportive evidence that lycopene may have chemopreventative effects against a broad range of cancers (lung, prostate, breast, and colon cancer). Dr. Wang and his colleagues, using the ferret model, investigated the hypothesis that lycopene inhibits lung carcinogenesis by upregulating IGFBP-3 as a molecular target and interrupting the signal transduction pathway of IGF-I as a mechanism for the chemopreventative effect of lycopene. He concluded that *1*) lycopene protects against smoke-induced lung carcinogenesis via upregulation of IGFBP-3, restoration of apoptosis, and inhibition of cell proliferation; and *2*) lycopene inhibits smoke-enhanced Bad phosphorylation in the lung in ferrets via induction of IGFBP-3.

In summary, the beneficial versus detrimental effects of lycopene may be related to the lycopene dose administered in vivo, the accumulation of lycopene in a specific organ, the interaction of lycopene with tobacco and alcohol, and the effects of lycopene metabolites or decomposition products on several important cellular signaling pathways and molecular targets. Ferrets provide a unique model for investigating lung cancer chemoprevention with lycopene. They are also useful for mechanistic studies to understand molecular changes that are relevant to lycopene metabolism and lung cancer in humans.

Discussion. A participant asked what percentage of animals had cancer at 6 mo. Dr. Wang responded that 6 of 12 ferrets developed grossly identifiable tumors, including both squamous cell carcinoma and adenocarcinoma, and 10 of 12 developed precancerous legions, including squamous dysplasia and atypical adnomatous hyperplasia.

The cleavage of lycopene into its metabolites could be a key direction for future research. A participant asked whether the shorter-chain metabolites act nonspecifically by disrupting membrane signaling or whether they bind a member of the steroid receptor superfamily. Dr. Wang replied that he is currently investigating the transactivation activity of a lycopene metabolite, apo-10′-lycopenoic acid, using the luciferase reporter gene and retinoic acid response element (RARE)/peroxisome proliferator-activated receptor element (PPARE). The overall goal is to understand why lycopene is able to induce IGFBP-3 expression. The presence of RARE in the promoter region of IGFBP-3 suggests that apo-lycopenoic acid may be mimicking retinoic acid to induce IGFBP-3. A comment was made that different oxidation products of lycopene have been shown to exhibit different agonist activities through retinoic acid receptor β and PPAR γ.

A participant asked whether the metabolites are as effective on a molar basis. Dr. Wang responded that this has not yet been studied and that the different outcomes between the lycopene and β-carotene studies may be due to the differences in the levels of carotenoids that accumulated in lung tissue. When 30 mg/d of supplemental β-carotene was administered, the concentration of β-carotene in the lungs in ferrets was 26 μmol/kg lung tissue, which was associated with increased development of lung squamous metaplasia induced by cigarette smoke exposure. When 60 mg/d of lycopene was administered, the concentration of lycopene in the lungs was only 1.2 μmol/kg lung tissue in ferrets, which caused no harmful effects and prevented the development of lung squamous metaplasia and cell proliferation induced by smoke exposure.

Lycopene versus Tomato Products: What Have We Learned from Rodent and Translational Studies?

Dr. Steven Clinton, Ohio State University

Prostate cancer rat and mouse models can be divided into 2 general categories: models of tumorigenesis (transplantable) versus models of de novo carcinogenesis (12). Only 3 studies on prostate carcinogenesis that evaluated dietary tomatoes or lycopene were found in the literature. There appears to be a trend in the study by Imaida et al. (13) toward carcinogenesis inhibition by lycopene (LycoRed) in the 3,2′-dimethyl-4-aminobiphenyl (DMAB) model, but not the 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) model. Dietary concentrations of lycopene used in these studies may be too low to achieve prostate concentrations similar to humans since absorption of carotenoids is substantially lower in rats and mice than in humans. This study also is limited by the statistical power and low overall incidence of cancer. Venkateswaran et al. (14) reported significant inhibition of prostate carcinogenesis by a combination of antioxidants (vitamin E, selenium, and lycopene) or by energy restriction using the Lady transgenic mouse model. The study design, however, does not allow for evaluation of the individual contributions of the antioxidants. In addition, the diet composition, supplement source, diet supplement content, and supplement intake are not defined in the paper, further compromising interpretation of the data. Boileau et al. (15) completed a larger study comparing the ability of lycopene or tomato products to inhibit *N*-methyl-*N*-nitrosourea (NMU)-induced rat prostate carcinogenesis. There was significant inhibition of carcinogenesis by supplemental tomato powder but not by lycopene. Dr. Clinton concluded that *1*) tomato powder may contain compounds, in addition to lycopene, that inhibit prostate carcinogenesis; *2*) modest diet restriction (20%) significantly reduced the risk of developing prostate cancer; and *3*) there were additive anticarcinogenic effects of tomato phytochemicals and diet restriction.

Although the relevance of any one model of rat prostate carcinogenesis to human disease is open to speculation, Dr. Clinton reported that their studies with the NMU model suggest that the tumors mimic many features of human carcinogenesis. A study by Liao et al. (16) found that the histopathology, tumor vascularity, proliferation rate, and intratumor heterogeneity of biomarker expression in rats recapitulate what is known about human prostate cancer. A second study (17) found that loss of androgen receptor expression, chromatin relaxation, nuclear morphometry, and activation of AKT in rat prostate tumors also mimic human disease. Of interest, one study by Wang et al. (18) found that tomato polyphenols may inhibit IGF-I signaling through the AKT pathway, which suggests one mechanism that may contribute to the observations from the NMU model.

There are many future directions for addressing the tomato and lycopene hypothesis in prostate carcinogenesis. Descriptive studies that evaluate important hypotheses derived from epidemiologic findings in well-characterized models are inno-

vative and critical for translation to future human intervention trials. Multiple models of carcinogenesis exhibiting differing biological characteristics should be evaluated. Dose-response studies are critical, and funding should be provided that allows investigators to complete experiments with the appropriate statistical power. Correlations between the dietary intake of lycopene (and other tomato compounds) and blood, tissue, and tumor concentrations should be explored. The role of lycopene metabolites, isomers, and other tomato carotenoids (e.g., phytofluene) must also be addressed. Finally, the emerging fields of genomics and proteomics are likely to provide critical insights into mechanisms of action.

Discussion. One participant asked how best to design studies, considering the heterogeneity of human prostate cancer, and whether laser capture microscopy should be used. In general, there is heterogeneity within a tumor, even in well-characterized animal models, as is true in human prostate cancer. Laser capture microscopy, in which the investigator can focus on a specific cell population, would add to the quality of studies. A transplantable system often provides more homogeneity in cell populations. Dr. Clinton recommended harvesting the tumors early in transplantable models, before they have had a chance to develop complex and heterogeneous intratumor biology. He also recommended that scientists spend as much time on study design as on data analysis to ensure high-quality gene array or proteomic data. With regard to the best models for "-omics" studies, the participant asked whether animal models or diseased/nondiseased tissues of human subjects should be used. Dr. Clinton replied that these are two distinct questions, and both are relevant and important to prostate cancer etiology and progression. In terms of diet, an understanding of the effect of nutrition on normal prostate cells will provide a foundation to understand the early steps of carcinogenesis. Studies in more advanced disease answer a different, but also legitimate, question regarding the ability of diet to influence tumor growth and progression. These studies may suggest a role for diet and nutrition as an adjunct to therapy.

Another participant asked about the use of mortality as an endpoint in Dr. Clinton's publication. Dr. Clinton responded that the vast majority of the animals died of prostate cancer over 1 y, which is substantially less than the life expectancy of a rat. Thus, survival in this model is a good surrogate for prostate carcinogenesis. If animals are killed at 25–30 wk, which substantially reduces costs, the majority of tumors are microscopic. If the study continues for 1 y, however, the cancers are significant and easily visualized and exhibit all of the histological characteristics of human prostate cancer. There are advantages and disadvantages of short versus longer studies.

Are There Adverse Effects of Lycopene Exposure?

Dr. Paula Trumbo, U.S. FDA

Dr. Trumbo presented a review of literature on the evidence for adverse effects of lycopene exposure (19). Animal studies evaluating the safety of lycopene can be classified using the following 6 categories: *1*) acute toxicity studies, *2*) subchronic and chronic safety studies, *3*) reproductive studies, *4*) genotoxicity studies, *5*) hepatic uptake studies, and 6) absorption, distribution, metabolism, and excretion studies. Most of the studies used crystalline lycopene—a synthetic form that is sensitive to light and oxygen, insoluble in water, and not suitable for commercial use. In addition, the lycopene formulations used in the majority of studies included ascorbic acid and/or vitamin E to prevent lycopene oxidation. These were small toxicology studies, with 6 to 10 animals/group.

Lycopene did not have any adverse effect in the acute toxicity study. The subchronic and chronic safety studies—which monitored factors such as body weight, hematology, blood chemistry, histology, food consumption, and motor activity—found no evidence of clinically significant adverse effects of lycopene (although some reported very minimal effects on food consumption, slightly suppressed appetite and growth rate, and slight changes in hematological measures and enzymes). The reproductive studies found no evidence of maternal or developmental toxicity with lycopene, and no signs of aborted pregnancies, malformations, or teratogenic effects. One genotoxicity study suggested that pure crystalline lycopene, when exposed to light and air, is degraded to compounds with some mutagenic activity. The other genotoxicity studies, however, found no increase in mutation frequency compared to controls, no increase in DNA damage, and no evidence of chromosome damage. Last, the hepatic uptake studies indicated that *1*) hepatic levels of lycopene are 2.5 times higher in rats fed a supplement versus tomato concentrate, with the highest concentrations in liver; and *2*) although lycopene deposits were observed in liver, they had no effect on liver pathology. In summary, these data suggest that there are no serious adverse effects of lycopene intake.

Rats accumulate lycopene in different tissues at levels similar to humans; thus, it appears that rats can be used as a reference for assessments of human health as well as for safety analyses. In terms of safety assessment, lycopene has no observed adverse effect level (NOAEL) of 3 g/(kg · d). According to NHANES-III data, the 50% level of lycopene intake from food is 5.2 mg/d, whereas the 99% level is 123 mg/d (7). Tomato products and watermelon provide 5 to 30 mg lycopene/serving and supplements contain 5 to 15 mg lycopene/capsule; therefore, it would require a high level of food or supplement consumption to reach the NOAEL or the 99th percentile of intake. No tolerable upper intake level was set for lycopene in the 2000 Institute of Medicine's Dietary Reference Intake Antioxidant Report because there are no observed adverse effects (7). In addition, the FDA had no questions in response to a notice that the use of synthetic lycopene as a food ingredient is generally recognized as safe.

Discussion. A recommendation was made to study the biologic activity of the breakdown products of lycopene. The breakdown products of β-carotene, for example, have been implicated in increased carcinogenesis.

Absorption of lycopene seems low. One participant asked what happens to the unabsorbed lycopene; particularly, what metabolism occurs in the colon. Dr. Trumbo replied that much is excreted in feces. Although a large amount of lycopene is excreted intact, some metabolites do occur.

A comment was made that one of the unanticipated problems in the β-carotene trials was that the carrier (oil) enhanced the absorption and bioavailability of β-carotene, resulting in β-carotene blood levels that were unexpected on the basis of absolute concentration. When designing lycopene clinical trials, attention should be paid to the lycopene formulation being administered and to changes in circulating levels, despite evidence that most lycopene is excreted.

Group Discussion 3: Defining Research Gaps and Setting Research Priorities

Moderator: *Cindy Davis, NCI, NIH*

An issue was raised about the applicability of studies in humans with high-fat diets since many animal diets are not high in fat. Dr. Davis suggested that transgenic or knockout

model systems be used to a greater extent in this kind of research. The Mouse Models of Human Cancers Consortium is a resource for mouse models. A comment was made that the P10 AKPT model could be an important animal model for prostate cancer. It was noted that the scientific community should not be limited to single models and that crossing models with other knockouts and transgene models could yield important clues about sites of action and reasons for variations in response.

Dr. Davis mentioned that within the past month a study was published that suggests that lycopene increases oxidative damage to fibroblasts in culture, and asked whether this had implications for human studies, particularly with regard to the use of lycopene in combination with radiation therapy and with regard to the potential for some of the adverse effects seen with β-carotene. A comment was made that in cell culture, the metabolic products of lycopene are exposed to oxygen, light, and other conditions that differ from conditions in the body. Another participant replied that the lack of lycopene toxicity is in the context of animals only; what happens in human patients is unknown. Lycopene should be tested with a focus on adverse effects in human patients (e.g., potential interactions with radiation, chemotherapy, and other drugs).

Dr. Davis asked whether there are more vulnerable individuals or populations that might be more susceptible to any potential adverse effects of lycopene. The comment was made that tolerable risk might be different for prostate cancer patients than for the general population. In human studies designed to collect toxicity data, a higher-risk group could be used if investigators are willing to tolerate more risk. Another participant recommended studying subjects with globally low levels of several carotenoids and antioxidants to determine whether a low dose of a factor such as lycopene has any protective effect. The epidemiological evidence suggests that in individuals with low carotenoid or antioxidant status, the benefit lies in achieving a nondeficient state. A recommendation was made to select subjects with low antioxidant status and to administer a moderate amount of lycopene to avoid the toxic amounts in the β-carotene trial.

With regard to therapy, an important question is whether lycopene levels predict different outcomes in prostate cancer patients. A study was cited that found that blood lycopene levels in prostate cancer patients were lower than in men without the disease. Because many men take dietary supplements, prediagnosis dietary measurement can be difficult, but this question should be explored.

It was mentioned that therapy is tumor regression, and that prevention is a deterrence to proliferation, spreading, and so on (all inclusive except for tumor regression). A comment was made that approaches to therapy and prevention likely differ with different cancers. Lung cancer patients may survive only 1 y postdiagnosis, whereas prostate cancer could take decades to develop. Even untreated, there may be no symptoms for 5–10 y. The biopsy defines prevention versus therapy of prostate cancer; the biology does not change before and after biopsy. Principles of prevention most likely have application in prostate cancer early after diagnosis. The continuum of the disease provides many opportunities to work with preventive strategy in a therapeutic setting. Even without tumor regression, slowing the growth rate and PSA velocity might have an effect on survival time.

The terms "lycopene" and "tomatoes" have been used interchangeably and likely should not be assumed to be identical. The matrix is important in the observed response. Instead of whole foods, investigators may need to study a bioactive component in food and then factors that modify the bioactive component. What factor in the matrix can modify lycopene, or is there a new component that should be explored? One participant responded that, from a nutrition perspective, it is not necessary to know which of 5 or 10 potentially bioactive components are active in tomatoes to conduct intervention studies. If a good hypothesis is generated, based on population and laboratory animal studies, it should be tested in clinical trials without a reduction to components.

An opposing viewpoint was offered that intervention studies often are conducted with lycopene, not tomatoes, by investigators who extrapolate and assume that lycopene is the bioactive factor. If in fact another chemical in tomatoes is the bioactive factor, the studies will fail. The participant responded that such reductionist thinking, assuming lycopene is the bioactive component, is the flawed logic. Research should be directed toward whole-food interventions. A comment was made that, if the rat studies have not provided sufficient evidence that lycopene prevents prostate cancer in the rat model, it is surprising that a mechanism is being discussed. There also may be synergistic effects of lycopene and other tomato components. One study showed that although β-carotene did not have an additional effect on the oxidative benefits of lycopene, there was an effect with vitamin E.

It is possible that tomatoes and other plants evolved carotenoids, over millions of years, as systems to protect the germ line, producing biologically active compounds for their own purposes. It would be naïve to assume, given how carotenoids came about, that these systems are dependent entirely on a single molecule. Plants most likely have evolved either redundant or interacting systems.

SESSION IV: WHAT HAVE WE LEARNED FROM CLINICAL TRIALS?

Moderator: *Dr. James Crowell, DCP, NCI*

A Randomized Pilot Clinical Trial of the Action (Independent Effects) of Isoflavones and Lycopene in Localized Prostate Cancer: Administration Prior to Radical Prostatectomy

Dr. Nagi Kumar, H. Lee Moffitt Cancer Center

Dr. Kumar summarized the primary objective of this study, to assess the effect of various supplemental doses of isoflavones or lycopene administered during the period prior to radical prostatectomy on intermediate endpoint biomarkers (IEBs) of prostate cancer risk in patients with clinically localized prostate cancer, compared to control patients receiving no supplements. The IEBs are *1*) biochemical (serum levels of free and total testosterone, estradiol, and PSA), *2*) proliferation (proliferating cell nuclear antigen Ki-67 from prostate tissue), and *3*) genetic/regulatory (apoptosis). The secondary objectives are *1*) to assess the association between changes in plasma isoflavone and lycopene levels and changes in the biochemical, proliferation, and genetic/regulatory markers; and *2*) to test the hypothesis that men with the greatest increase in plasma levels of circulating isoflavones and lycopene will demonstrate the greatest reduction in IEBs of prostate cancer risk. This is a controlled, randomized pilot study; the cohort is prostate cancer patients in the presurgical phase; the duration of intervention is from 4 to 6 wk; and the agents are isoflavones in 40-, 60-, and 80-mg doses and lycopene in 15-, 30-, and 45-mg doses. Sixty-seven subjects are participating in the pilot trial. There are no results at this time.

Lycopene for Androgen-Independent Prostate Cancer

Dr. Aminah Jatoi, Mayo Clinic

Dr. Jatoi presented the study design of a Phase II trial conducted by the North Central Cancer Treatment Group, an NCI-funded national cooperative group based in Rochester, Minnesota. The trial will build on prevention data, investigate whether cancer patients benefit from voluntary supplementation with agents such as lycopene, and expand the limited therapeutic options in androgen-independent prostate cancer. Preliminary clinical data also support further study of lycopene for the treatment of cancer. In a study by Ansari and Gupta (20), for example, 20 androgen-independent prostate cancer patients received 10 mg/d of lycopene. The investigators found a 35% response rate (described as a drop in PSA level) in the cohort.

In this trial, 40 patients with asymptomatic, androgen-independent prostate cancer were treated with 15 mg of lycopene twice a day, administered as a tomato foodstuff. The primary endpoint is a 50% confirmed response in PSA level decrease. Accrual is complete, and the data are being analyzed.

The 30-mg/d lycopene dose was chosen based on preliminary efficacy data from other studies. The trial relies on a historical control group, based on a previously published and peer-reviewed study by Dr. Jatoi with the same eligibility criteria, which found that the agent being tested had no efficacy. Compliance was not monitored because the patients are highly motivated (i.e., they are not anticipating cancer, they already have hormone-refractory cancer). In addition, even without compliance data, the trial still should provide patients with some preliminary guidance as to whether to take lycopene.

Discussion. A participant questioned the logic and ethics of a trial without a control group. Interpreting the outcome or making inferences for patient care will be difficult if not impossible. Dr. Jatoi replied that Phase II studies often are conducted in a cancer setting. The data can be interpreted because the natural history of hormone-refractory prostate cancer is known. Dr. Jatoi tried to include a control arm in an earlier trial, but the NCI questioned the ethics of a nontreatment arm as part of a trial in patients with active disease. It also is possible that the agent in the control arm (e.g., soy) would have therapeutic efficacy, leading to equivalence between arms. Another participant added that, in medical oncology, Phase II clinical trials often are conducted to collect preliminary data for Phase III clinical trials, not to make recommendations to the public. Furthermore, there is no effective treatment for the patients in this study, so any nontoxic agent should be explored.

A suggestion was made to design trials with similar agents and control arms, so that the data could potentially be merged. It was also noted that when exploring indications of toxicity, it is helpful to monitor side effects in the control arm.

It was suggested that a decrease in the slope of PSA level rise might be useful to examine. Dr. Jatoi might observe a decrease in PSA doubling time, which will prolong the patients' survival. The chosen endpoint, a decrease in PSA level, sets up the trial for failure. Dr. Jatoi responded that, although the rate of rise in PSA level was not chosen as an endpoint, this could be examined as well.

What Are Some of Our Current NCI Clinical Lycopene Studies?

Dr. Keith Rodvold, University of Illinois—Chicago

Dr. Rodvold first described an ongoing Phase I multiple-dose pharmacokinetic study of lycopene, delivered in a well-defined food-based lycopene delivery system (tomato paste–oil mixture), in patients at increased risk for developing prostate cancer. The objectives are *1*) to define the toxicity and safety of a chronic (3-mo) schedule of lycopene administered in a food-based delivery system at 3 different doses/d, *2*) to define the pharmacokinetics and tissue distribution of lycopene administered to patients at increased risk for developing prostate cancer, and *3*) to characterize surrogate endpoint biomarkers in the peripheral blood, buccal cells, and prostate that will provide evidence of biological activity relevant to a chemoprevention effect. The study participants, who must satisfy a list of inclusion criteria (e.g., a biopsy that fails to reveal prostate cancer), were administered either a 15-, 46.5-, or 78-mg lycopene dose. Study endpoints include *1*) serum lycopene concentrations (including other carotenoids and lipid-soluble vitamins) and pharmacokinetics at 1 and 3 mo of treatment, *2*) tissue distribution of lycopene (oral mucosa and prostate tissue), *3*) safety and toxicity of 3 mo of treatment, and *4*) modulation of surrogate endpoint biomarkers [DNA oxidative stress in blood, oral mucosa, and prostate tissue; serum PSA (total, free, and percentage free); proliferation and apoptosis in prostate tissue; and IGF-1 and modulation of the prostate].

Dr. Rodvold described a second ongoing trial—a Phase I single-dose pharmacokinetic study of the dietary supplement lycopene delivered in capsule form to healthy male volunteers between 18 and 45 y of age. The objectives are *1*) to define the toxicity and safety of a single dose of a lycopene food supplement packaged in gelatin capsules, *2*) to define the pharmacokinetics of a single dose of lycopene administered as a capsule to a group of healthy male subjects, and *3*) to define the dose range of lycopene formulation to be used in the 3-mo multiple dose study, based on the pharmacokinetic and toxicity data resulting from this Phase I study. The study participants, who were required to satisfy a list of inclusion criteria (e.g., a prestudy serum lycopene concentration of $<$700 nmol/L), were administered a 10-, 30-, 60-, 90-, or 120-mg lycopene dose. Study endpoints include *1*) toxicity and safety of a single dose of a lycopene food supplement packaged in gelatin capsules, *2*) pharmacokinetics of a single dose of lycopene administered as a capsule (including total, *cis*, and *trans* isomers of lycopene as well as chylomicron lycopene during the first 12 h), and *3*) concentrations of carotenoids and lipid-soluble vitamins.

How Do Intermediate Endpoint Biomarkers Respond to Lycopene in Men with Prostate Cancer or Benign Prostate Hyperplasia?

Dr. Richard B. van Breemen, University of Illinois

Dr. van Breemen first described several in vitro studies carried out with prostate cancer cell lines to investigate lycopene effects and uptake (21). He concluded that *1*) analytical methods development facilitated both in vitro and in vivo studies, *2*) lycopene exhibited only minor inhibition of human prostate cancer cell proliferation in some cell lines (e.g., LNCaP), and *3*) all prostate cancer cells took up lycopene from the cell culture media at different rates. Proteomics studies of the effect of lycopene on prostate cancer cells are in progress.

Dr. van Breemen also described the design of a 21-d Phase II clinical trial conducted with 116 men to investigate the effects of lycopene on biomarkers in men with benign prostate hyperplasia (BPH) and cancer. The study will test the following hypotheses: *1*) Lycopene prevents DNA oxidation both in vitro and in vivo (i.e., Does lycopene prevent formation of multiple DNA oxidation products, or only certain products? Is

lycopene a prooxidant? Can in vitro experiments predict in vivo effects of lycopene?). *2*) Oral administration of lycopene results in increased concentration in the prostate. *3*) Serum lycopene reflects prostate levels. *4*) Lycopene administration reduces serum PSA. Sixty men diagnosed with BPH and 60 men with prostate cancer were recruited. Subjects were randomly assigned to 2 groups administered pills containing either lycopene (30 mg) or placebo for 21 d in a double-blind study. Blood samples were obtained at the beginning (baseline) and at the end of the intervention period, and prostate tissue was obtained at the end of the study from either resected tissue or biopsy. Total lycopene was measured in blood samples and prostate tissue using negative ion atmospheric pressure chemical ionization (APCI) liquid chromatography (LC)-mass spectrometry (MS)-MS with a C_{18} high performance liquid chromatography column; 2 DNA oxidation products (8-oxo-dG, 8-oxo-dA) were measured in peripheral blood white blood cells and prostate tissue using electrospray LC-MS-MS; lipid peroxidation in plasma was measured using APCI LC-MS-MS; and PSA levels were measured in blood before and after intervention using ELISA. The data from this trial currently are being analyzed.

Dr. van Breemen also provided a summary of the preliminary dietary intervention study (22). Men with elevated serum PSA levels were recruited into the tomato sauce/whole-foods–based study prior to radical prostatectomy. Biopsy and blood samples were obtained for lycopene and DNA oxidation measurements. Thirty-two men with stage T1 or T2 adenocarcinoma of the prostate completed the study. Subjects consumed 200 g of tomato sauce in pasta dishes for 21 d (30 mg lycopene/d). Total lycopene was measured in serum and prostate tissue obtained at the time of radical prostatectomy, 8-oxo-dG was measured in peripheral blood white blood cells and prostate tissue, and PSA levels were measured in serum before and after intervention. The results suggest that *1*) lycopene in serum increased 2-fold, but lycopene levels in prostate tissue increased 3-fold; *2*) DNA oxidation (measured as 8-oxo-dG) decreased 21% in leukocytes; *3*) DNA oxidation decreased 28% in prostate tissue; and *4*) total PSA levels in serum decreased ~20%. These results are consistent with epidemiological data showing an inverse correlation between tomato consumption and risk of prostate cancer.

Discussion. A participant asked why Dr. van Breemen failed to observe an effect of lycopene in cell culture, when many studies in the literature show profound inhibitory effects and changes in outcomes. Dr. van Breemen replied that he has published relatively little on cell culture work because he has observed variation from one technician to another as well as variation with the method of lycopene delivery (liposomes versus beadlets), with the use of organic solvents, and with tissue culture conditions and cell passage number. A comment was made that it may be more difficult to obtain cell culture data relevant to in vivo conditions with carotenoids than with other agents (e.g., isoflavones or polyphenols). In several cell culture studies in the literature, lycopene alone failed to inhibit cell growth significantly, but inhibition was observed in conjunction with other agents (e.g., phytoene and phytofluene).

It was suggested that the biological effects of lycopene are mediated by its metabolites; thus, if the cell cannot convert lycopene to its metabolites, there may be no effect. A participant asked whether Dr. van Breemen checked for the presence of lycopene metabolites in the cell culture study. Dr. van Breemen replied that he had checked for this. Because lycopene is under such unusually high oxidative stress in the cell culture system, and because many lycopene degradation products are so short lived, there are no definitive quantitative data to report.

The *cis* and *trans* forms of lycopene appear to be interconverting, so Dr. van Breemen will report total lycopene in the study he is currently conducting. A participant commented that it is unclear whether other investigators are measuring physiological levels of *cis* and *trans* isomers or the equilibrium state in serum samples that cannot be stabilized. A counterpoint was offered that although lycopene is unstable in an organic solution, there are other carotenoids, lipids, etc., present in a serum sample that tend to stabilize and preserve the isomeric distribution.

Another participant asked whether cross-study comparisons can be made, given the methodological problems with these studies. Dr. van Breemen responded that the lycopene levels are high enough and the analytical methods reliable enough, whether based on electrochemical detection, absorbance detection, or MS, to compare lycopene levels in tissue and blood between studies. With regard to measuring other outcomes, PSA measurements were done with ELISA and should provide similar results between studies. The DNA oxidation measurements were difficult, however, and variation has been found in measuring similar systems across laboratories. DNA damage in cells is controllable and repairable, and unrepaired damage is on the order of parts per billion. Some have suggested that a better measure of oxidative stress would be levels of 8-oxo-dG and other excised and excreted nucleosides in urine, where they are stable. This would not be specific to oxidative stress in the prostate, however; it is a whole-body oxidative stress measurement.

What Are the Changes in Molecular and Cell Morphology Markers in Men with High-Grade Prostatic Intraepithelial Neoplasia after Lycopene Supplementation?

Dr. Peter Gann, Northwestern University

Phase III trials are slow and expensive. With the knowledge currently available, investigators cannot yet design high-quality Phase III trials to test the lycopene/tomato hypotheses. It is important to investigate ways to optimize tissue-based biomarkers in small Phase II prevention trials.

Dr. Gann described the potential designs for Phase II trials and their limitations (23). There are 3 Phase II design options: preradical prostatectomy (abundant and fresh tissue, short exposure, difficulty comparing the biopsy and surgical sample); prebiopsy (many patients, shorter exposure, high endpoint variance); and biopsy/rebiopsy (3–12-mo exposure, low endpoint variance, fewer patients). Only about one-third of patients with initial high-grade prostatic intraepithelial neoplasia (HGPIN) have detectable HGPIN on a repeat biopsy. As a result, HGPIN would require large sample sizes for adequate statistical power and is not an ideal Phase II endpoint.

The molecular and cytometric characteristics of high-risk "normal" (i.e., histologically benign) tissue can provide crucial IEBs for prevention trials as well as useful clinical predictors for risk in patients with negative biopsies. Dr. Gann uses 4 techniques—differential expression by progressive compartments, comparison of "supernormal" versus "normal" tissue, comparison of near versus far, and a case-control study with previous negative biopsies—to identify field effects. Biomarker candidates include α-methylacyl-CoA racemase (AMACR) and several protein expression markers [Ki67 and Mcm2 (proliferation), activated caspase 3 and Bcl2 (apoptosis)]. Some broad research agenda suggestions for intermediate tissue biomarkers in Phase II prevention trials are the following: *1*) agree on necessary steps in initial development of tissue bi-

omarkers, including statistical concerns (i.e., sources of variation); 2) expand the types of assays that can be done on small samples of formalin-fixed, paraffin-embedded tissue; 3) achieve greater precision in immunohistochemical and cytometric assays via standardized digital image analysis; 4) understand how to identify field effects in normal tissue (especially in nonaccessible organs); 5) identify the molecular and genetic characteristics of "high-risk normal" tissue; and 6) agree on how to achieve ultimate epidemiological validation of intermediate tissue markers and the extent to which such validation is needed.

Discussion

One participant asked what is meant by HGPIN "going away" in two-thirds of the cases upon repeat biopsy. Dr. Gann responded that HGPIN could still be present but is not being detected because of sampling error.

Another participant asked about nuclear morphometry and whether grade is a summary of some of the 44 parameters. Dr. Gann replied that he used a multivariate multigrade score, a combination of selected features from the set of 44, with appropriate weights derived from a discriminate analysis model. A different model is being used now. The model selection process is complicated because many of the 44 features are tunable, which can affect the results.

What Biomarkers May Be Most Useful for Predicting a Response to Tomatoes in Men with Prostate Cancer?

Dr. Phyllis Bowen, University of Illinois—Chicago

There are many different types of biomarkers, including exposure markers, biomarkers of cancer, function-related biomarkers, tumor markers, biomarkers of tumor burden, and biomarkers of tumor–host interaction. Dr. Bowen focused on surrogate endpoint biomarkers (SEBs), defined as "measurable biological processes or molecules that are closely linked to the progression pathway to invasive cancer, and which undergo change in concert with neoplastic regression" (24). Questions relevant to identifying SEBs for lycopene and tomato products include the following: *1)* What are the candidate bioactive substances in tomato? *2)* What pathways are modulated by the major substances in tomato? *3)* What SEBs have been identified on these pathways? *4)* What additional promising SEBs are worthy of further evaluation for lycopene/tomato efficacy?

SEBs closest to the true endpoint of cancer are likely to be the most predictive of lycopene efficacy. Genomic and proteomic patterns of expression, as well as DNA methylation patterns, are promising, but a paradigm of clear "cutpoints" must be determined for the efficient design of Phase II trials based on sensitivity and specificity. The literature currently lacks population studies that test promising SEBs and a presentation of continuous variables that allows for the calculation of cutpoint estimates with a high level of specificity.

The many proposed pathways for lycopene action in prostate health include antioxidant/prooxidant function, inhibition of IGF-I transduction, inhibition of androgen activation and signaling, inhibition of cell cycle progression/apoptosis, inhibition of inflammation, inhibition of phase II enzymes, and an increase in gap-junction communication. Besides PSA, only DNA damage and IGF pathways are promising, based on the existence of biomarkers with which sensitivity and specificity can be assessed. Lycopene and quercetin show a moderate ability to modulate various carcinogenic pathways at physiologically feasible concentrations. Lycopene appears to modulate several pathways, so SEBs on each pathway should be included in Phase II trials.

Once an attributable proportion is known for lycopene and other substances in tomato, consideration should be given to combinations with either other natural substances or existing therapies. There is a pressing need to evaluate the safety and efficacy of lycopene/tomato supplements combined with radiation and androgen oblation. Specificity can be increased by combining SEBs.

Discussion. A participant commented that there are no prospective data in the literature, either from animals or humans, on whether DNA oxidative markers are helpful. DNA repair mechanisms are very active, so it is not known whether DNA oxidative markers make a difference in vivo.

The comment was made that it is misleading to measure DNA damage in patients who already have cancer. The predictive value of a test cannot be estimated by comparing subjects who have cancer to those without cancer. A better approach would be to determine who, among the disease-free subjects with DNA damage, eventually will develop cancer. It is possible that the subjects have high levels of DNA damage because they have cancer, rather than that they developed cancer because of the DNA damage. The selection of surrogate endpoints must be based on validated methods. A critical area of research is the examination of biomarkers in disease-free subjects.

How Strong Is the Evidence That Lycopene Supplementation Can Modify Biomarkers of Oxidative Damage and DNA Repair in Human Lymphocytes and Buccal Cells?

Dr. Sian Astley, Institute of Food Research, Norwich, England

Several assays are available to measure DNA damage (e.g., base loss or modification, replication error, interstrand cross links, DNA-protein cross links, strand breaks) and DNA repair (e.g., mismatch repair, base excision repair, nucleotide excision repair, double strand break repair, damage by-pass) (25). The uncertainties, limitations, and inconsistencies involved with research on DNA damage and repair should be addressed. Furthermore, there is no evidence to support the hypothesis that DNA damage and repair in lymphocytes reflect tissue response elsewhere. Lymphocytes most likely differ from other tissues with regard to exposure (oxidative stress and proposed protective compounds), cellular metabolic rate, and available resources (small dNTP pool).

In general, tomatoes are the major source of lycopene. Different tomato varieties have different compositions, and tomatoes contain many biologically active compounds other than lycopene (minerals and vitamins, flavonoids, and phenolic acids). Daily tomato consumption has been found to reduce the risk of cancer of the respiratory and digestive tracts, stomach, and lung; insufficient data are available in other areas. There is no differentiation between raw and cooked tomatoes, except in prostate cancer (cooked greater than raw), and there is little agreement on the effects of tomatoes versus lycopene. The epidemiology is not well-founded; problems include researcher bias and a lack of high-quality food composition data. Measuring lycopene quantitatively has been difficult, and there is a poor understanding of the bioavailability, absorption, digestion, metabolism, and excretion of lycopene in the human body.

Conclusions being drawn from in vitro lycopene experiments appear to be specific to the system under study (dependent on the type of liposome mix, cells, carotenoids, etc.). The extent of uptake into cells, for example, has been found to be

carotenoid specific and dose responsive. Dr. Astley emphasized that the Comet assay provides a semiquantitative measure of the number of single strand breaks and alkali-labile sites, not of DNA damage per se. Repair may not occur as quickly in lymphocytes as in other cells, resulting in the accumulation of single strand breaks as indirect indicators of ongoing repair. Thus, does an observed increase in single strand breaks indicate damage or ongoing repair? Dr. Astley found >60 in vivo lycopene studies in the literature that show a DNA-related "response" to lycopene or tomatoes. Discrepancies, such as no response in peripheral blood lymphocytes or differences between surrogate and target tissues, can be explained by differences in exposure (e.g., plasma concentration or tissue distribution) or other physiological causes.

In summary, the evidence that lycopene supplementation can modify biomarkers of oxidative damage and DNA repair in human lymphocytes is only as good as the study design and biomarkers used. Lycopene supplementation in vitro is associated with changes in markers of DNA damage and possibly indirectly with DNA repair. Where plasma concentration does not change, lycopene supplementation appears to have no effect on peripheral blood lymphocyte DNA damage or repair. Lycopene supplementation, where target cell or plasma concentration is increased, is associated consistently with decreased DNA damage or increased repair in the target tissue or the surrogate. Future work on this topic should be more critical and more holistic.

Discussion. If the U-shaped curve of single strand breaks exists, does this indicate that high exposures to lycopene create safety concerns? Dr. Astley replied that this depends on the interpretation of the U-shaped curves. An interpretation of the single strand breaks as damage would suggest potentially harmful effects. Alternatively, if it is true that these cells do not have the resources to make repairs, the curve may represent not damage, but the overlapping effects of damage and repair activities. Firm statements cannot be made until damage and repair activities are separated.

A participant commented that there are 3 questions to address: *1*) In what ways do the epidemiological studies fit or not fit with one another? *2*) What are the likely bioactive compounds in tomatoes? *3*) What are the mechanisms of the different pathways? Dr. Astley stated that epidemiologists must be provided with better data. A comment was made that the rate-limiting step in the discipline first must be determined. The difficulty in assessing diet over time, for example, could be a more significant issue than the food composition data provided to epidemiologists. In addition, it is important to evaluate critically the extent to which biomarker studies can be used as evidence that either lycopene or tomatoes are associated with risk of prostate cancer.

Group Discussion 4: Defining Research Gaps and Setting Research Priorities

Moderator: *John Milner, DCP, NCI*

Dr. Milner suggested that with respect to the specificity of response, from the information presented so far, the focus is best on the effects of tomatoes and not limited solely to lycopene. If these observations are valid, studies may be needed that examine various components within the tomato (e.g., flavonoids). A comment was made that, in an intervention with a processed food or pill that contains a wide variety of the active ingredients present in tomatoes, it may not be critical to identify the specific entities that are active.

Another approach would be to develop a tomato with the appropriate and consistent content. Another participant stated that it seems necessary to study components of tomatoes, not whole tomatoes, to obtain research support. The critical component of any application is a high-quality, hypothesis-driven, probing series of studies—whether focused on tomatoes or components of tomatoes. Regardless of whether tomatoes or individual tomato components are effective as modifiers of prostate cancer, all options should be explored. More classical pharmacognosy approaches should be used in fractionating the tomato and conducting bioassay-guided fractionation to identify new active compounds. The NCI funds several natural product cancer prevention studies that take advantage of this approach. Another topic for future research is synergy among tomato components.

A comment was made that, although the lycopene versus tomato issue can be debated at this meeting, the public is asking what scientists have offered in terms of dietary methods to reduce cancer risk. The "reductionist" and "whole-food" approaches are not necessarily antagonistic. The whole-food approach might provide a proof of principle more quickly. Research on tomato constituents, however, also is important to elucidate the mechanisms of action. The field of diet and cancer has not had notable breakthroughs in recent years, and it is important for the scientific community to recognize that there is a need to move quickly and develop some convincing successes. The public already believes, whether factually based or not, that lycopene has a benefit in prostate cancer. It is important to identify via Program Announcements, Requests for Applications, and intramural research which populations will benefit (if any) and how to identify those populations.

Epidemiologists have tried to isolate one component of the diet and determine whether that component is associated with cancer risk by adjusting for all other factors. For the major components of the diet—fish, red meat, milk, and so on—this approach might be successful. With smaller dietary components, however, measurements are more difficult and the matrix has a greater effect. A more global approach is needed that examines the component as a part of the matrix (i.e., more sophisticated, complex studies with more biomarkers; biological samples from before the occurrence of disease; and so on). In the past, epidemiologists have attempted to eliminate the background to determine if one factor has an effect above a "foggy" background. Future studies should examine these factors simultaneously (i.e., examine small, minute regulations of normal metabolic processes). Another participant commented that, despite the challenges, the epidemiological evidence is suggestive of a weak association, which might be stronger in a small, susceptible group. Investigators are more likely to find an effect in a population with low lycopene levels and a genetic or other type of susceptibility.

Several drug models already exist and are available (e.g., the chemoprevention model, drug discovery agent development model, NMU model, Dunning transplantable tumor model). A participant recommended working with these models in an interdisciplinary way. Prospective human intervention trials could be conducted with one manufactured agent that mimics the natural tomato but has less variation in its components. Furthermore, a wide variety of biomarkers should be developed based on molecular targets, mechanisms of action, and disease endpoints. A participant offered a counterpoint that a uniform range of components must be delivered by the producers of natural tomatoes to meet the quality demanded by consumers. Synergy among tomato components remains an unknown; thus, the scientific community must ensure that the high-lycopene tomato has a positive and not a negative effect.

Populations consuming the lowest amounts of tomatoes might be particularly vulnerable. More attention to genetic polymorphisms or factors associated with stress might provide clues about who will benefit most. Polymorphisms associated with DNA repair, generation and removal of free radicals, etc. may be needed to truly identify vulnerable individuals.

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